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to the effect that the corolla arises as a distinct whorl of organs which are formed later than the inner sepals and receive bundles which leave the axis at a higher point. In the same way the long stamens are shown to be a distinct whorl and in no sense appendages of the shorter ones.

Chodat and Lendner agree in nearly all points with the view of Klein, and argue that the seemingly bicarpellary gynœcium of the normal Cruciferæ is in reality due to the union of four carpellary members. This view is based chiefly upon the course of the bundles in certain anomalous three- or four-carpelled specimens of *Cheiranthus cheiri* L. It is scarcely necessary to say that a conclusion from these rather doubtful premises must be accepted with all due caution.

B. L. R.

**Zinsser on Root Tubercles of Leguminosæ.** — In *Jahrb. f. wiss. Bot.*, Bd. xxx, Heft 4, pp. 423–452, may be found an interesting paper by O. Zinsser on the root tubercles of the Leguminosæ. This paper contradicts some of the statements of Frank, Gonnermann, Laurent, etc., especially the statement that the root tubercle organism occurs outside of the tubercles in various parts of the plant. This work was done in the Botanical Institute at Leipzig. The following are some of the more important statements:

1. Seeds of all sorts of leguminous plants were washed in sterile water, soaked fifteen minutes in water containing mercuric chloride (1 : 1000), washed again thoroughly in sterile water, planted in sterile earth, covered with cotton-plugged sterile bell jars, and watered with sterile water. The plants which grew from these seeds were under observation eight to twelve weeks, but in no case did any tubercles form on their roots. If, however, the contents of root tubercles of these same plants was added to the earth, tubercles developed on the roots in most cases in fourteen days. The author believes with Prazmowski that Dr. Frank's diametrically opposite results were due to the fact that he did not succeed in freeing his seeds from adhering surface organisms. So far as could be detected, the sublimate treatment did not in any way injure the plants.

2. Other aërial parts and roots destitute of tubercles were then tested in various ways for the occurrence of the germ:

(*a*) Approved staining methods, *e.g.*, carbol fuchsin, alkaline methylen blue, gentian violet in anilin water, etc., were used on sections, but in no case could bacteria be demonstrated in the tissues.

(*b*) The author washed out the contents of tubercles in water, injected this into growing stems or roots of seedlings, and after a lapse of from four to six weeks washed their surface thoroughly with sterile water, bruised them in a sterile porcelain mortar, mixed thoroughly with sterile earth, planted therein the sterilized seeds, and obtained root tubercles in three weeks on the roots of almost every plant. This would indicate that, if the germs are normally present in the stems and other non-tuberculous parts of leguminous plants, the bruised tissues added to sterile earth ought to infect the roots of seedlings grown therein. Consequently, roots, stems, leaves, and leafstalks of *Phaseolus vulgaris*, *P. multiflorus*, *Vicia sativa*, *V. faba*, *Pisum sativum*, *Ervum lens*, and *Lupinus albus* were tested in this way after cutting away the superficial portions with sterile knives, but in no case did any root tubercles appear.

(*c*) After isolating the root tubercle organism from four plants (*Phaseolus multiflorus*, *Pisum sativum*, *Lupinus albus*, and *Vicia faba*) and determining that the cultures were able to produce root tubercles on the specified plants, and would remain alive for a long time when injected into parts above ground, attempts were made to isolate these organisms from other parts of the same plants, using four different media, *viz.*: (1) sterilized hydrant water; (2) water, meat extract, peptone, and sugar in the following proportions,—100, 0.5, 0.5, 3.0; (3) decoction of the plant mixed with washings of earth; (4) decoction of the plant, asparagin, and sugar in the following proportions,—100, 0.25, 0.5. These nutrient solutions were sterilized in small Erlenmeyer flasks. The inoculations were made in a room rendered as free as possible of floating germs by the introduction of vapor of water. The selected, above-ground tissues were washed for a long time and very carefully in sterile water, cut into small pieces with flamed shears, and put carefully into the flasks, part of which were exposed to the air and the rest subjected to an atmosphere from which the oxygen was removed as completely as possible. Bacterial growths appeared in only a few of the flasks, and none of these produced any root tubercles when added to the sterile earth in which the seeds were grown. This experiment was repeated on roots free from tubercles with the same negative result.

(*d*) When sterilized seeds had sufficiently germinated, the roots were put through a tiny opening in the bottom of a glass pot, and then the bottom of the pot was filled with a mixture of sterilized gypsum and water, so that the middle part of the roots was cemented fast. The part of the root above the partition of gypsum was then covered with sterile earth and watered with sterile water, the part below being put into ordinary earth mixed with bruised tubercles. The whole was then protected from air infection by a glass cover. Root tubercles formed on this lower part of the root, but none were found on the root and rootlets grown in the sterile earth. Cultures from the part of the roots grown in the sterile earth and from the parts above ground yielded negative results; bruised portions added to sterile soil also failed to cause any root tubercles on the roots of seedlings grown therein. The reverse experiment was tried, *i.e.*, using infected soil in the upper chamber and sterile soil below. In this case tubercles formed on the roots in the upper chamber and not on those in the lower one. Cultures and soil infections from these lower roots yielded negative results. These experiments indicate that the germs cannot migrate from the tubercles to other parts of the plants.

3. Mr. Zinsser determined with considerable care the length of time the root tubercle organisms of *Phaseolus multiflorus* are able to live when injected into germinating seeds, young roots, young and old stems, leafstalks, etc., of various legumes. His conclusions do not differ much from results obtained by numerous observers working with all sorts of organisms non-pathogenic to plants. In other words, they were able to retain vitality and make a feeble growth for a variable period, usually two to ten weeks, but only in close proximity to the place of insertion, the germs being injected by means of a Pravaz syringe. The infectious material was derived from pure cultures and also directly from the tubercles.

4. Water washings from the root tubercles of three different legumes (*P. multiflorus*, *V. faba*, and *L. albus*) were injected into a great variety of plants (forty-two species of many different orders), but after eight weeks the organisms were not to be found in any of them either at the point of insertion or two centimeters away. Moreover, on the roots of none of these plants could tubercles be induced, although abundant washings of root tubercles were added to the earth. Pure cultures and tubercle washings from *Phaseolus multiflorus*, *P. vulgaris*, *Vicia faba*, *Lupinus albus*, and *Pisum sativum* were also injected into the callus on cut branches of locust, poplar, and willow, but after forty days no living bacteria were to be found therein.

5. The author also tried the behavior in plant tissues of a variety of micro-organisms non-pathogenic to plants, with results much like the preceding and entirely confirmatory of what we already know, namely, that many saprophytes and animal parasites are able to live

in plants for some weeks (three to ten or more in Mr. Zinsser's experiments), and frequently make a feeble growth, especially when injected in large numbers. Mr. Zinsser's experiments include ten bacteria and were made on no less than fifty of the higher plants, Leguminosæ included, unquestionably involving an enormous amount of hard work. Pure cultures were always used. These were diluted with water and injected by means of a Pravaz syringe. In all cases the growth, if any, was restricted to the immediate vicinity of the puncture. On examination, bacteria were found only in the injured cells and in the intercellular spaces, never in the uninjured living cells as a result of their own activities. The length of time the bacteria were able to live in the tissues varied with the plants, and was different for different micro-organisms. Even the most resistant spores (those of *B. subtilis*, *B. prodigiosus*, *B. megaterium*, and *B. anthracis*) died in the plant tissues inside of eighty-six days.

6. The author did not succeed in growing the genuine tubercle bacilli on agar or gelatin prepared according to Beÿerinck's direction and Gonnermann's. On such media numerous germs were obtained from the tubercles after sterilizing their surface, but they were non-infectious, and must be considered as associate forms or secondary growths (see Beÿerinck's statements). The tubercles were sterilized by soaking ten minutes in 1 : 1000 sublimate solution, or by washing thoroughly in sterile water, soaking for a few minutes in alcohol and then burning this off. After numerous failures, the true germ was finally isolated on Winogradsky's silicate jelly. In Petri dishes on this medium at the end of eight days the colonies were small and white, grew well in the juice of the host plants, and produced tubercles on their roots. Living colonies were found in anærobic cultures at the end of three weeks. The microbe is about 1 $\mu$ long, and actively motile. Neither spores nor flagella could be demonstrated. The colonies obtained from the tubercles of different plants looked just alike, and addition of plant juices to the silicate jelly did not in any way change the appearance of these colonies. The organisms were also morphologically indistinguishable.

7. Flasks of water, sugar, magnesium sulphate, and potassium phosphate in the following proportions, 100, 5.0, 0.1, 0.1, were inoculated, put in a dark place, and aërated with air from which by passage through potash water and then through sulphuric acid all nitrogen compounds were removed. After forty-nine days these

flasks were opened. No living bacteria were present in any of them, nor were the fluids able to produce tubercles when added to the roots of plants growing in sterile earth. Under these conditions the germs were not able to assimilate free nitrogen.

8. It is not clear in just what way the tubercles originate. Their production is due to the action of specific organisms, but these are not always capable of causing them, as the frequent failures showed. The author was not able to produce them by direct inoculations, not even in the tissues of young roots and stems. He thinks that possibly infection takes place only through young root hairs. Contrary to Laurent, the time of year makes no difference; neither does the age of the plant, as Nobbe has also shown, since tubercles were obtained both on the roots of seedlings and on those of well-developed plants. Gain's observation that infections are more numerous in a damp soil is confirmed.

ERWIN F. SMITH.

**Recent Studies of Asarum.** — The wild gingers of the Eastern and Middle United States, concerning the specific definition of which some doubt has long been felt, form the subject of papers by Bicknell in the *Bulletin of the Torrey Botanical Club* for November last, Ashe in the first part of the current volume of the *Journal of the Elisha Mitchell Scientific Society*, and Kraemer in the *American Journal of Pharmacy* for March. In commenting on some of these papers in the *Journal of Botany* for March, James Britten and Edmund Baker analyze the synonymy of certain of the species and call rather emphatic attention to the desirability of consulting types in serious systematic work. Some slight bibliographic confusion is likely to result from the publication of Mr. Ashe's paper in separate form long enough before the number of the *Journal* containing it was issued to enable him to revise the latter into quite a different article. T.

**Combs's Flora of Santa Clara Province, Cuba.** — The island of Cuba is one of considerable interest to the botanist, as is shown by the rich collections made by many early explorers. In recent years, however, the region seems to have been neglected. We have before us a contribution of considerable length devoted to the flora of Cienfuegos, province of Santa Clara, by Robert Combs.[1] The author enumerates 713 species, of which *Caesalpinia cubensis*, *Acacia*

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[1] Plants Collected in the District of Cienfuegos, Province of Santa Clara, Cuba, in 1895–1896. *Trans. Acad. Sci. of St. Louis*, 7: 393–491, pls. 30–39, one map, 1897. (Contributions Botanical Department, Iowa State College of Agric. and Mechanic Arts, No. 7.)